Emulsion Properties of Pseudo-Ceramide PC104/Water/Polyoxyethylene Cholesteryl Ether and Polyoxyethylene Cetyl Ether Mixtures.

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ABSTACT

The formation of emulsions and micelles in water/ceramide PC104/CholEO_{20}/$C_{16}EO_{20}$ and water/ceramide PC104/CholEO_{20} mixtures was investigated through the phase behavior studies. The phase diagrams showed the existence of micelle and emulsion regions in both systems. The mixed surfactant system (CholEO_{20}/$C_{16}EO_{20}$) showed the wider micellar and emulsion regions than the single surfactant system (CholEO_{20}). From FT-IR measurements, it was found that the polyoxyethylene (POE) groups of surfactants formed the hydrogen bonds with amido carbonyl group in ceramide PC104. This result indicated that the hydrophilic part (EO) of surfactants could stabilize the lamellar structure and emulsion of ceramide PC104. The mixed surfactant system (CholEO_{20}/$C_{16}EO_{20}$) resulted in the smaller emulsion droplet size due to the effect of curvature at the interface, thus further increasing emulsion stability. With the penetration of $C_{16}EO_{20}$ into the interfacial layer of surfactants in emulsion, the curvature of the interface might be altered for the formation of smaller emulsion droplets. The mixed surfactant system could incorporate up to 4 wt.% of ceramide PC104 into emulsion more than single surfactant system.

Keywords: Ceramide PC104, Lamellar structure, Liquid crystalline emulsification, Hydrogen bond, Fluidity,

INTRODUCTION

The stratum corneum lipids (SCL), which exist in the intercellular space of the stratum corneum, are known to provide an epidermal barrier against water loss as well as protection against environmental, chemical, and biological stress [1,2,3]. It was reported that the removal of stratum

corneum (SC) resulted in an approximate 100-fold increase of the epidermal water loss [4,5].

The effective barrier function of stratum corneum (SC) has been attributed to a highly ordered structure of lipid bilayers observed in the intercellular space SC [3,6,7]. Therefore, the mechanisms for the self-assembly of the lipid bilayer as well as their physicochemical properties have been a subject of considerable interest [8,9,10]. The SCL lamellae are composed mainly of ceramides, free fatty acids, cholesterol, and cholesteryl esters, and the presence of ceramides has been suggested to be the basis for the structural organization of SCL in bilayers [6,11].

Ceramides constitute of ~40% of human SC lipids [12]. They are considered to play a critical role in the barrier property of SC [13]. The ceramide is composed of a long-chain alcohol to which a long-chain fatty acid is linked by an amide bond. Ceramides have both a polar headgroup and a nonpolar tail. Two or three free hydroxyl groups and the amide group form the relatively small polar head-group. The content of free hydroxyl groups and the presence of an amide group enable ceramide to act as a hydrogen bond donor and acceptor [14]. This allows ceramides to interact with neighboring molecules, forming an intermolecular hydrogen bonding network [15]. FT-IR and FT Raman spectroscopy were employed to study the molecular structure of the SC [16]. Ceramides, whose hydrophobic tails are straight and almost entirely saturated [12,17], can form bilayers with closely packed interiors [13]. These properties of ceramides would be suited for the epidermal barrier function

A decreased level of ceramides in stratum corneum of atopic dermatitis has been found [18]. It has also been suggested that the application of small amount of ceramides may be effective to the dry skin dermatitis [19].

Forslind has recently developed a structural model of the skin barrier, which describes stratum corneum function in terms of lipid organization at the molecular level. Drawing from the fields of research dermatology and general membrane biophysics, it has been proposed that the lipids of the SC are organized in large domains of crystalline/gel phase lipids bounded by regions of liquid crystalline lipids. This is called the domain mosaic model of the skin barrier [20,21]. Elias suggested that the presence of ceramides and spingolipid is the basis for the structural organization of SCL in bilayer [3,12]. Wertz et al. showed that ceramides could take part in the bilayer formation and fatty acids were essential for this to occur [13]. Recently our preliminary study showed that, the region of each phase was extended or contracted depending on the concentration of pseudo ceramide PC104 in octanoic acid. On the contrary to the region of L2, regions of lamellar phase and L1 phase were expanded. We explained the bilayer forming ability of ceramide PC104 on the basis of concentration of ceramide PC104 at interface [22].

Even though ceramides constitute the major fraction of SC, they exist in nature only in a small

quantity. To make them commercially available for the dermatological application, Kim et al. recently synthesized a pseudoceramide, named ceramide 16 (ceramide PC104). They examined the dermatological applications of ceramide 16 (ceramide PC 104) to the dry skin and damaged skin, and found that the synthetic ceramide exhibited an excellent water-retaining properties. The synthetic pseudo ceramide has been suggested to be useful as a skin moisturizer and the restoration of skin barrier damaged [23]. Many other pseudo ceramides were also reported for the application in cosmetics and foods.

In the present study, we have investigated the bilayer-forming ability of the mixtures containing the water, ceramide PC 104, $CholEO_{20}$ and $C_{16}EO_{20}$. We used pseudo-ceramide PC104 synthesized by Kim and co-workers [23]. The partial phase diagrams of ceramide PC104 with $CholEO_{20}$ were constructed and the effects of $C_{16}EO_{20}$ on the phase behaviors have been investigated. The emulsion droplet sizes of ceramide PC104/$CholEO_{20}$/Water and ceramide PC104/$CholEO_{20}$/$C_{16}EO_{20}$/water were also measured to investigate the effect of $C_{16}EO_{20}$ at the interface. The additional purpose of this study was to add ceramide PC104 at active concentrations into an emulsion that would prevent its conversion to its γ-crystal structure.

EXPERIMENTAL

Materials

The pseudo-ceramide 1,3bis(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane (PC 104) was obtained from Pacific Co (Seoul, Korea), the molecular structure is shown in figure 1. Polyoxyethylene cetyl ether ($C_{16}EO_n$: n is the number of ethylene oxide) and polyoxyethylene cholesteryl ether ($CholEO_n$) with average number of ethylene oxide unit of 20 were purchased from Nihon Emulsion Co. and used without further purification.

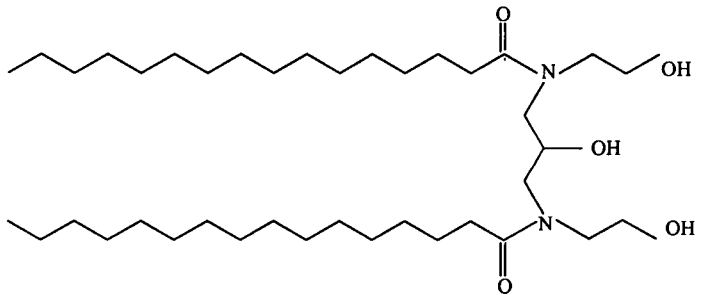


Figure 1. Molecular structure of ceramide PC104

Methods

Partial phase diagram:

The partial phase diagrams were constructed using a traditional titration method. Appropriate amounts of ceramide PC 104, $CholEO_{20}$ and $C_{16}EO_{20}$ were weighted into glass vials. The mixing ratio of $CholEO_{20}$ to $C_{16}EO_{20}$ was 3:1 by molar ratio. The sealed glass vials were placed in the incubator at 30℃. To initial two-component system, which was invariably a homogeneous paste, the third component (water) was added by means of syringe so as to incrementally increase the weight percentage of that component through the course of the whole experiments. The homogeneity of the three components was achieved by heating the mixture up to 75℃, and stirring (vortex) for 10-15 minutes. After this procedure, samples were kept in the incubator at 30℃ for a few days. Identification of phases was performed by observation of the samples by visual inspection, against scattered light, and between crossed polarizers to check the homogeneity and birefringence of samples.

FT-IR spectroscopy:

Ceramides are amphiphilic molecules and have a polar head group. Thus, IR spectroscopy makes it possible to elucidate the structural information from the polar region of ceramide. The absorption wavelengths of C=O stretching vibration associated with a C-N stretching in ceramide PC104 were measured. These wavelengths reflected the extent of interaction among amido carbonyl groups of ceramide PC104. The infrared measurements were carried out with MANGNA-IR 760 SPECTROMETER (Nicolet Inc., USA).

Structural analysis:

The structures and states of the lipids were identified by X-ray diffraction with a GADDS small angle X-ray diffractometer (BRUKER, Germany). Cu Kα radiation (λ=1.542Å) was used in X-ray diffractometers.

Fluorescence analysis:

The fluidities of the hydrophobic areas of the emulsion were evaluated by measuring the anisotropic fluorescence using DPH (Diphenylhexatriene) as indicator, which was added into the emulsion. The probe was excited at 360 nm wavelength, while the emission was measured at 420 nm wavelength. Steady-state anisotropic fluorescence, r_{ss}, was determined according to

$$r_{ss} = \frac{I_{II} - I_{\perp}G}{I_{II} + 2I_{\perp}G}$$

where I_{II} and $I_{\perp}$ are emission intensities when emission polarizer is oriented parallel and

perpendicular to excitation polarizer, respectively. Fluorescence intensity was measured on a LS 50B fluorescence spectrometer (Perkin-Elmer,Norwalk, CN).

Emulsification procedure:

Lipid mixture of ceramide PC104/CholEO_{20} or ceramide PC104/$C_{16}EO_{20}$/CholEO_{20} was prepared at 80℃. To form emulsion, the distilled water was slowly added to lipid mixtures while stirring at room temperature. The droplet size was measured using Zetasizer 3000HS (Malvern instruments Ltd, UK).

RESULTS AND DISCUSSION

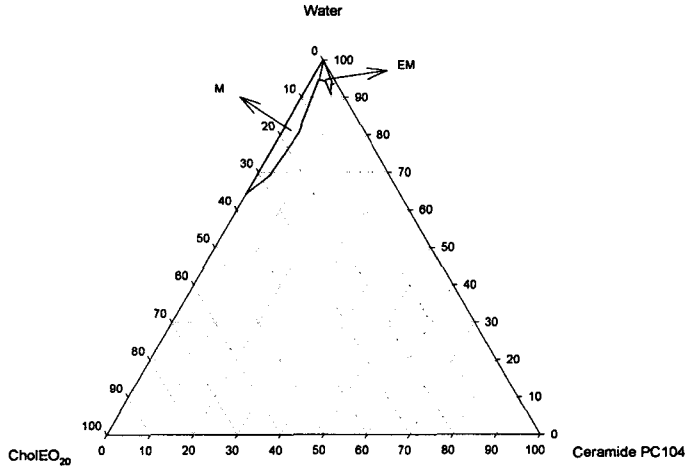
Ternary Phase diagrams:

In the present work phase diagrams were constructed for the following two systems of ceramide PC104/CholEO_{20}/water and ceramide PC104/CholEO_{20}/$C_{16}EO_{20}$/water. The phase diagrams at 30℃ are depicted in figures 2. The diagrams showed the existence of Micelle (M) and Emulsion (EM) regions in all systems. The ceramide PC104 alone cannot form micelle and emulsion phase since it is too hydrophobic. Since the ceramide PC104 exhibits a very strong hydrophobicity due to two long hydrocarbon chains, as well as the small polar head groups, there is a close balance between hydrophilic and lipophilic properties, which is inclined toward the lipophilic properties. Thus, ceramide PC104 has strong tendency to form liquid crystals, notably of lamellar structure. M (Micelle) and EM (Emulsion) phases were observed in the narrow regions for all phase diagrams. This domain was identified by visual inspection, and inspected through crossed polarizer and polarization microscopy, respectively. The very viscous gel-like phases were observed in all other regions except M (Micelle) and EM (Emulsion) in figure 2. Thus, it was very difficult to ascertain the exact phase whether phases were lamellar or solid phases.

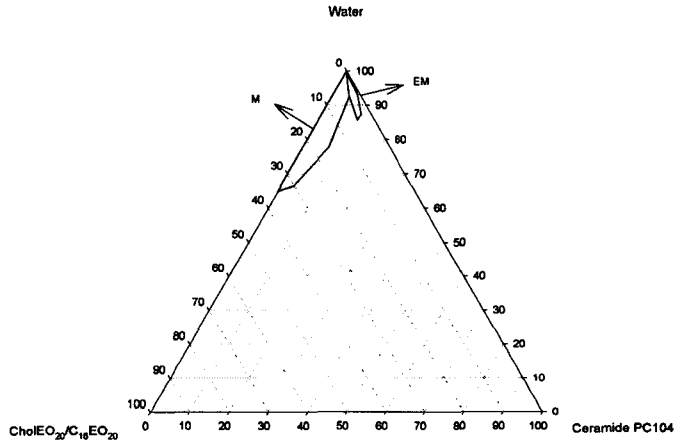
Comparisons between the single surfactant system (CholEO_{20}) and the mixed surfactant system (CholEO_{20}/$C_{16}EO_{20}$) show that there is an increase in the micelle domain and in the emulsion region in the mixed surfactant system. The maximum concentration of ceramide PC104 in emulsion studies on single surfactant system was 6.5 wt.%, while it was 10.2 wt% in mixed surfactant system. Therefore, the ceramide PC104 was stably incorporated into emulsion in the mixed surfactant system up to 4 wt.% more than in the single surfactant system. The partition of straight hydrocarbon chain of $C_{16}EO_{20}$ at interface can affect the curvature of the interface [24]. The more partition of $C_{16}EO_{20}$ in the interfacial layer of micelle and emulsion droplet, the easier formation of M and EM region at the ceramide PC104-based phase diagrams.

XRD, FT-IR absorption spectra and droplet size of emulsion have been investigated to clarify the effect of $C_{16}EO_{20}$ and CholEO_{20} in the formation of stable LC and emulsion containing ceramide

PC104.



(a)



(b)

Figure 2. Partial ternary phase diagrams of (a) Ceramide PC104/CholEO_{20}/water and (b) Ceramide PC104/CholEO_{20}/$C_{16}EO_{20}$. EM: Emulsion, M: Micellar Phase.

Effect of hydrophilic group:

From the FT-IR study, it was found that the polar groups of ceramide PC104 were sensitive to hydrogen bonding and/or polarity effects. The strong hydrogen bond played a decisive role in the stabilization of lamellar structure and emulsion containing ceramide PC104 [25,26]. Iwai et al. have reported that stearic acid contributed to the stability of a lamellar structure through hydrogen bonds between the carboxyl group of stearic acid and the amidocarbonyl group of ceramide [25]. But a mixture of ceramide and stearic acid has a low water binding capacity, because their small polar

head groups have little affinity for water. Thus the ceramide/stearic acid lamellar structure does not allow emulsification. However, as the water binding capacity is increased, increasing amounts of water can be incorporated into the lamellar structure. The more water is sandwiched in the inter-lamellar spaces, the more the lamellar layers separate and form emulsions. This phenomenon is known as "liquid crystalline emulsification"[27].

Incorporation of large amounts of water into the lamellar structure can be archived by adding surfactants ($CholEO_{20}$, $C_{16}EO_{20}$) with a high water binding capacity. At the same time, these molecules have to be able to form hydrogen bonds with ceramide to prevent its crystallization. Thus, we performed infrared (IR) spectroscopy measurements to elucidate the interaction of between polar head group of ceramide PC104 and hydrophilic portion of surfactants ($CholEO_{20}$,$C_{16}EO_{20}$). The difference in absorption wavelength of these mixtures reflects the degree of interaction.

An intermolecular hydrogen bond between polyoxyethylene group (POE) and the amido carbonyl group of ceramide PC104 is shown in figure 3. It was found that amido carbonyl group peak occurred at 1632 cm^{-1} in the mixture of ceramide PC104/surfactants and at 1616 cm^{-1} when ceramide PC 104/surfactants/water mixture was in its most stable state. Polyoxyethylene (POE) part of surfactants was found to form the hydrogen bonds with the amido carbonyl group of ceramide PC104. Also, it is suggested that the role played by water molecules is essential for strong interaction between ceramide PC104 and polyoxyethylene. These results indicated that the hydrophilic part (EO) of surfactants could stabilize the lamellar structure and emulsion of ceramide PC104.

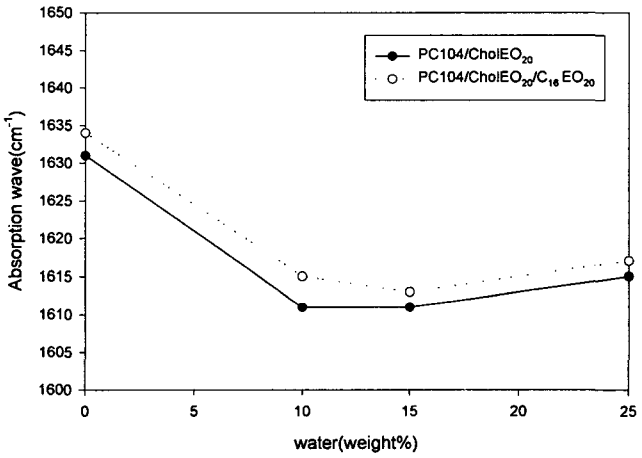


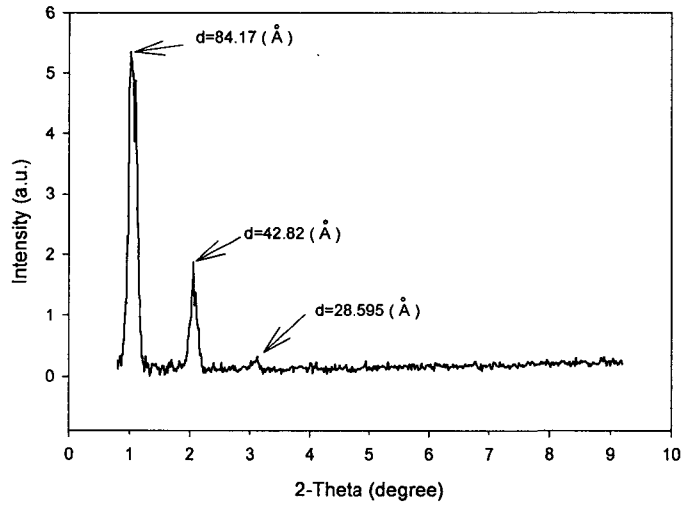
Figure 3. Maximum FT-IR peaks of amido carbonyl group of ceramide PC-104 in lamellar structure with the variation of water contents.

(●) Ceramide PC104/$CholEO_{20}$ (1/1: molar ratio)

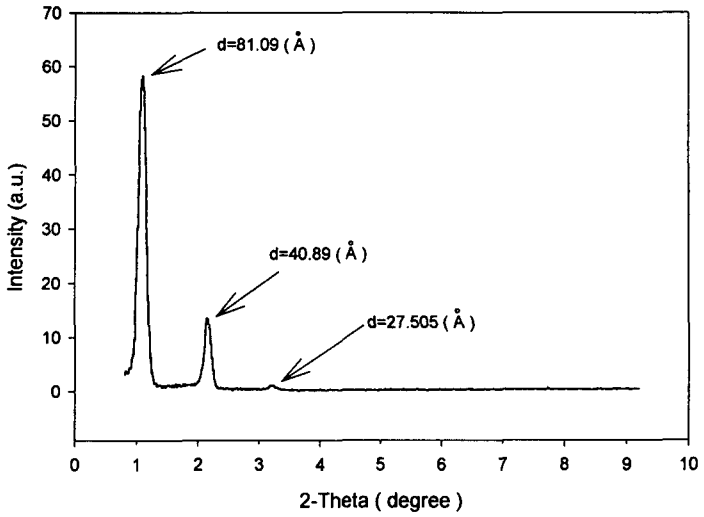
(○) Ceramide PC104/$CholEO_{20}$/$C_{16}EO_{20}$ (1/0.75/0.25:molar ratio)

Effect of Hydrophobic group:

While hydrogen bonds between hydrophilic groups are necessary for the stability of a lamellar structure, the hydrophobic groups of the molecule likewise contribute to the stability of the lamellar structure. Short carboxylic acids like formic acid or acetic acid cannot form a lamellar structure with pseudo ceramide due to the absence of alkyl chain [25]. The $CholEO_{20}$ and $C_{16}EO_{20}$ mixed with ceramide PC104 resulted in a stable lamellar structure as shown in figure 4. Usually a cholesteryl substitute induces the fluidity of the hydrophobic portion of the lamellar structure, also known as cholesteric effect [28]. The $C_{16}EO_{20}$ and $CholEO_{20}$ mixed with ceramide PC104 resulted in a stable lamellar structure, as the hydrophilic portion of surfactants could form hydrogen bonds with the amidocarbonyl group of ceramide PC104 as proven by figure 3. The hydrocarbon chains of $C_{16}EO_{20}$ mixed $CholEO_{20}$/ceramide PC104 mixture in the interfacial film are densely packed, and the interfacial film are more rigid than that without $C_{16}EO_{20}$ [29]. This increase in packing lowers the attractive force among the hydrocarbon chains, and hence, decreases of the amount of bound water in the bilayer [30]. The holding capacity of cermide PC104/$CholEO_{20}$ lamellar structure was as high as that of the one based on cermide PC104/$CholEO_{20}$/$C_{16}EO_{20}$ as shown in figure 5.



(a)



(b)

Figure 4. The diffraction patterns of the lamellar structure.

(a) Ceramide PC104/CholEO_{20}/water (25.5%/59.5%/15%: wt %)

(b)Ceramide PC104/CholEO_{20}/$C_{16}EO_{20}$/water (25.5%/45.9%/13.6%/15%: wt%)

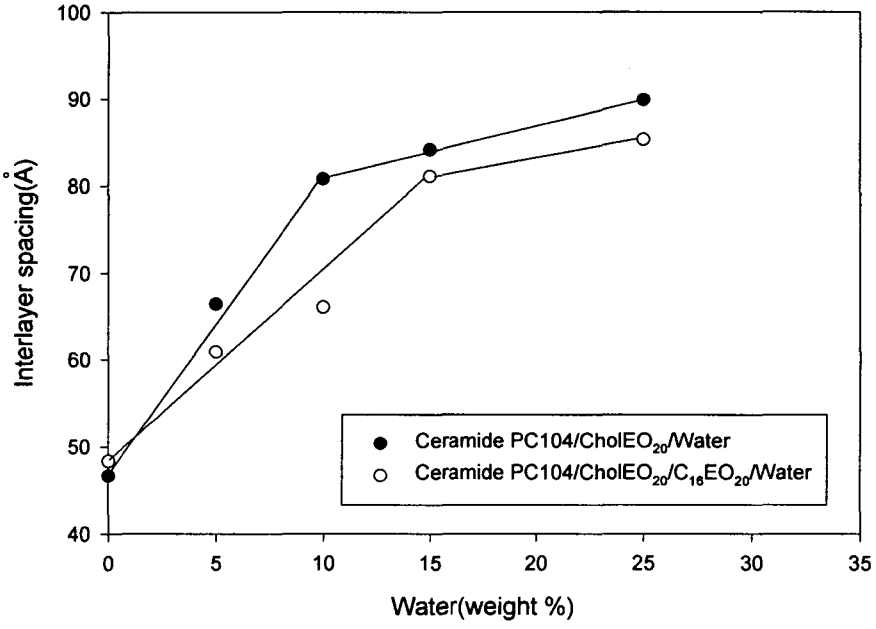


Figure 5. Incorporation of water into the interlamellar spaces of the lamellar structure.

(●) Ceramide PC104/CholEO_{20}(1/1: molar ratio)

(○) Ceramide PC104/CholEO_{20}/$C_{16}EO_{20}$(1/0.75/0.25:molar ratio)

Characteristics of the emulsion:

The lamellar structure of ceramide PC104/CholEO$_{20}$ and ceramide PC104/CholEO$_{20}$/ C$_{16}$EO$_{20}$ mixtures were converted into emulsion by adding more than about 80 wt% of water. Emulsions have been investigated in their size. Figure 6 shows that the emulsion droplet sizes depend on the amount of ceramide PC104. Emulsions have small and uniform droplets, a particle size of 700-1080 nm for ceramide PC104/CholEO$_{20}$ emulsions and 415-670 nm for ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$. The emulsion has small and homogeneous droplets, because it passes through a liquid crystalline phase during the process of emulsification [25]. The maximum concentrations of ceramide PC104 in emulsion were investigated by using the phase diagram of ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$/water system in figure 2. It was possible to stably incorporate up to 10.2 wt% of ceramide PC104 into emulsion. The emulsion droplet size for the ceramide PC104/CholEO$_{20}$ system was larger than that of the ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$ system. Iwai et al. have reported that the difference in emulsion size between SLE (pseudo-ceramide)/CholEO$_n$ and SLE/C$_{18}$EO$_n$ mixtures is due to a difference in the fluidity of the hydrophobic portions of the microemulsions. The fluidity of hydrophobic parts follows the order of SLE/C$_{18}$EO$_n$< SLE/CholEO$_n$, and the emulsion droplet size of SLE/CholEO$_n$ is smaller than that of SLE/C$_{18}$EO$_n$ [26]. As shown in Table 1, the fluidity of the hydrophobic portion of ceramide PC104/CholEO$_{20}$ was higher than that of ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$. But the emulsion droplet size of ceramide PC104/ CholEO$_{20}$/C$_{16}$EO$_{20}$/water mixture is smaller than that of ceramide PC104/ CholEO$_{20}$/water mixture. The different phenomenon might occur in the present system. It was suspected that the partition of straight chain of C$_{16}$EO$_{20}$ at interface could affect the curvature of the interface. The more partition of C$_{16}$EO$_{20}$ at interfacial layer of emulsion, the smaller emulsion formation than single surfactant system (ceramide PC104/CholEO$_{20}$) without C$_{16}$EO$_{20}$. Moreover, the fluidity of two systems (ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$ and ceramide PC104/CholEO$_{20}$) displayed the slight difference (Table 1). It was suggested that the droplet size of emulsion was more influenced by the curvature of interfacial layer than the fluidity of hydrophobic part in this system.

Table 1. Fluorescence anisotropy of emulsion depending on the hydrophobic group of the surfactant.

Composition	Fluorescence anisotropy (r_{ss})
Ceramide PC104/CholEO$_{20}$/Water (3.75%/2.5%/93.75%: wt%)	0.107
Ceramide PC104/CholEO$_{20}$/C$_{16}$EO$_{20}$/Water (3.75%/1.94%/0.56%/93.74%: wt%)	0.132

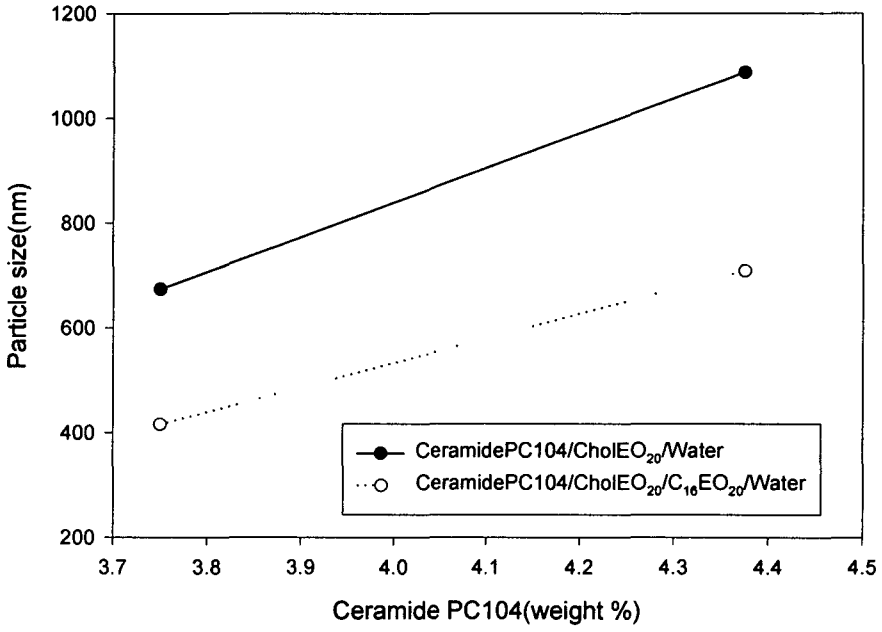


Figure 6. Average droplet sizes of emulsions depending on the amount of ceramide PC104.

(●) Ceramide PC104/$CholEO_{20}$/water

(3.75%/2.5%/93.75%, 4.38%/1.88%/93.74%: wt%)

(○) Ceramide PC104/$CholEO_{20}$/$C_{16}EO_{20}$/water

(3.75%/1.94%/0.56%/93.74%, 4.38%/1.44%/0.44%/93.74%: wt%)

CONCLUSIONS

The mixed surfactant system ($CholEO_{20}$/$C_{16}EO_{20}$) yielded the wider micellar domain and the emulsion region than the single surfactant system ($CholEO_{20}$). The ceramide PC104 was successfully incorporated into an emulsion at concentrations of up to maximum 10.2 wt.% in the mixed surfactant system. The interaction between the polar groups of surfactants ($C_{16}EO_{20}$, $CholEO_{20}$) and ceramide PC104 prevented the ceramide PC104 molecules from being crystallized out. Also, the role played by water molecules was essential for strong interaction between hydrophilic part of ceramide PC104 and polyoxyethylene. The mixed surfactant system ($CholEO_{20}$/$C_{16}EO_{20}$) resulted in smaller emulsion droplet size due to its effect on the curvature of the interface, thus further increasing the emulsion stability. With the penetration of $C_{16}EO_{20}$ into the interfacial layer of emulsion, the curvature of the interface was altered for the formation of smaller emulsion droplets.

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